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Modified plasma clearance technique using nonradioactive iothalamate for measuring GFR

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Glomerular filtration rate (GFR) is believed to be the overall index of renal function in health and disease. And the renal clearance of inulin (C_{In}) obtained during constant intravenous infusion has long been accepted as the gold standard of GFR measurement. Because of a number of technical difficulties inherent in the assay of inulin concentration in urine and plasma, its usefulness in clinical practice is limited.

Iothalamate, a urographic contrast medium which behaves and is excreted in a fashion similar to inulin, has been accepted as a good filtration marker [1–3]. The renal clearance of iothalamate is not much more simplified than that of inulin. But its measurement is used instead of inulin in the assessment of GFR.

Whether inulin or iothalamate is used, the measurement of renal clearance requires proper collection and measurement of an accurate short-timed urine specimen. Therefore improper urine collection sometimes leads to error in GFR measurement.

To overcome this disadvantage, GFR is alternatively determined as the total plasma clearances calculated from serial measurement of plasma concentrations after a single rapid intravenous injection, where urine collection is not necessary [4, 5]. The total plasma clearance should be equal to glomerular filtration rate, if the compounds would be excreted solely by glomerular filtration without tubular secretion or reabsorption. The conventional method of the total plasma clearances, however, usually needs multiple blood samples to calculate from the slope of the plasma disappearance curve. The plasma disappearance curve is generally separated into two compartments: the early phase reflects the distribution of the marker and the late phase reflects the excretion of the marker. The latter is closely related with the clearance rate [6]. So, the late phase of the plasma disappearance curve would offer a simple and good approximation of GFR, and it would conquer the disadvantages of the renal clearances and total plasma clearances, that is, timed urine collections and multiple blood sampling, respectively.

The aim of this investigation was to study whether GFR could be estimated by the modified plasma clearance technique to calculate only from the late phase of the plasma decline at 60, 90, and 120 minutes, without urine collection, when iothalamate was used as filtration marker and high-performance liquid chromatography (HPLC) system as the analyzing method.

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Methods

Subjects

Twenty-four patients, seventeen male and seven female, aged 17 to 72 years (median 47 years), weighing 47 to 80 kg (median 62 kg) with chronic renal disease and wide range of renal function (inulin clearance: C_{In} 10 to 130 ml/min), and three healthy male volunteers, aged 31 to 34 years (median 32 years), weighing 60 to 67 kg (median 63 kg) were studied. The clinical diagnoses of patients were chronic glomerulonephritis (21), diabetic nephropathy (2), and polycystic kidney disease (1). Three patients had moderate edema due to nephrotic syndrome. None had lower urinary tract obstruction. All studies were performed in Osaka University Hospital. Subjects with a history of allergy to iodine were excluded from entry. Informed consent was obtained from all patients and volunteers.

Protocol

After an overnight fast, subjects were given orally 300 ml of water before the study. Subjects rested supine, but were allowed to stand up during voiding. A polyethylene catheter was inserted into a cubital vein in both arms. Depending on GFR and the body weight, a 25% inulin solution was continuously infused from t = −45 minutes. In eight patients, a 20% para-aminohippuric acid (PAH) solution was simultaneously infused. At t = 0 min, 1 ml of a 30% iothalamate (Conray®) was injected and the catheter was flushed through with 5 ml of a 0.9% NaCl solution. Blood samples were drawn from the opposite arm at 10, 30, 60, 90 and 120 minutes. In only one healthy volunteer, blood samples were drawn at 2, 5, 10, 20, 30, 60, 90 and 120 minutes. At t = 15 minutes, subjects voided and clearance timing began. Four 30-minute clearance periods were then performed. Urine samples were collected by voluntary voiding.

Iothalamate and PAH were determined by HPLC system which consisted of Shimadzu LC-6A liquid chromatograph, Shimadzu SPD-6A UV spectrophotometric detector (Shimadzu

Co., Kyoto, Japan) and reverse-phase column YMC-Pack A312 S-5 (6 mm × 150 mm; Yamamura Chemical Laboratories Co., Kyoto, Japan). The mobile phase consisted of methanol and 0.1 mol/liter NaH_2PO_4 (15:85, vol/vol). The flow rate was 1.0 ml/min. Samples were quantified by UV absorption at 245 nm relative to a standard curve. Serum samples were precipitated with four volume of 0.64 mol/liter perchloric acid and centrifuged. Urine samples were diluted 20 times with deionized water prior to addition of perchloric acid. An aliquot of the supernatant (5 μl) was subsequently injected onto the column.

The concentration of inulin was measured by anthrone method [7]. The concentration of creatinine in serum and urine was measured by the alkaline picrate colorimetric method [8]. PAH was measured by colorimetric method, too [9].

Calculations

The renal clearances of inulin, creatinine, and iothalamate were calculated using the standard formula: C = UV/P, in which V is the urine volume per minute, and U and P are the urine and plasma concentrations. And the average of four 30-minute clearance periods was regarded as the renal clearance of the subject.

The decline of the plasma level after an intravenous injection of iothalamate could divide into two phases and the feasibility of using a two-compartment model to describe the elimination of iothalamate was investigated by using the following formula:

$$Cp = Ae^{-\alpha t} + Be^{-\beta t}$$

where Cp is the plasma concentration; α,β, the hybrid rate constant; A,B, the zero-time intercept; and t is time.

The first phase (α) includes the distribution of drug from the central compartment, such as the intravascular space, into the second compartment, such as the extravascular space. After a certain time, the graph moves into a log/linear phase (β) (Fig. 1a). This log/linear phase (β) represents elimination from the central compartment. The hybrid rate constant, β, and the clearance rate constant are closely related. The elimination of iothalamate is considered to take place only from the central compartment. Therefore, the rate of elimination at equilibrium is governed by the amount of iothalamate in the central compartment, and the clearance is calculated from the slope of the linear terminal part (β).

The hybrid rate constant, β, and the zero-time intercept, B, were calculated from the slope of late decline at three time points (60, 90 and 120 min) by using the following formula:

$$Log_{10}Cp = B - \beta t \text{ (Fig. 1b)}$$

The modified plasma clearances of iothalamate (C_{Iot}-M) were calculated using the following formula:

$$C_{Iot}\text{-M} = \frac{D \times \beta \times \log_e 10}{1.34 \times 10^B}$$

where D is the initial amount of iothalamate; 1.34 is the correlation factor, approximately calculated from the intravascular space and distribution volume [10].

Results

Representative chromatograms of serum and urine samples are shown in Figure 2. Iothalamate was eluted as a single peak at 6.0 minutes, and no interfering peaks were observed in the serum and urine sample blanks. The assay was liner within the wide range of concentrations of iothalamate (1 to 100 mg/liter). And the coefficient of variation of repeated measures of iothalamate was 2.1%. In our HPLC system, para-aminohippuric acid (PAH) could be simultaneously determined (retention time: 4.0 min). The detection limit for PAH was approximately from 1 mg/liter to 100 mg/liter. The PAH values obtained by our HPLC method were well consistent with those obtained by the p-dimethylaminocinnamaldehide colorimetric method of Yatzidis [9]. The difference between the values obtained by the two independent methods was within ±2.5%.

The renal clearances were determined in 23 subjects, because one subject could not void punctually. The renal clearances of creatinine and iothalamate (C_{Cr}, C_{Iot}) were compared with that of inulin. The slopes with zero intercept of C_{Cr} versus C_{In} and C_{Iot} versus C_{In} were 1.23 ± 0.01 (r = 0.99) and 1.05 ± 0.01 (r = 0.98), respectively.

The serial plasma concentrations of iothalamate in one healthy volunteer after an intravenous injection are shown in Figure 1c. This plasma disappearance curve was similar to the graph of the two compartment model, shown in Figure 1a. From t = 60 minutes the graph moved into a log/linear phase. The modified plasma clearance of iothalamate compared with the renal clearances of iothalamate and inulin are shown in Figure 3. The slopes with zero intercept of C_{Iot}-M versus C_{Iot}, and C_{Iot}-M versus C_{In} were 0.99 ± 0.01 (r = 0.98) and 1.05 ± 0.01 (r = 0.99), respectively.

In this study side effects including nausea, vomiting, and urticaria were not observed.

Discussion

In the present study, we employed a new method, modified plasma clearance, to determine glomerular filtration rate. This method employed non-radioactive iothalamate and required no constant infusion, timed urine collection, nor multiple blood sampling. The concentration of iothalamate was easy and accurate to measure by using HPLC.

Radioactive iothalamate has become universally accepted as an accurate marker for determining GFR [1–3]. But the use of radioisotopes requires complicated handling of storage and disposal of contaminated waste, and has an additional disadvantage of radiation exposure of the bladder wall and gonads, and a risk of uptake by thyroid gland by the patient. A sterile, pyrogen-free aqueous preparation of radioactive iothalamate is not necessarily available except for the United States. Recently, analytical methods for measuring iothalamate by HPLC have been developed [10, 11]. However, we developed a new analytical method for measurement of iothalamate. This method offers the additional advantage of being able to simultaneously measure PAH. Some colorimetric procedures of PAH assay may be positive for a variety of para-amino aromatic compounds, whereas our HPLC assay was linear within wide range of concentrations of PAH, and no interference was found in plasma and urine. So, our HPLC method seems to have a greater advantage than colorimetric assays, especially in high

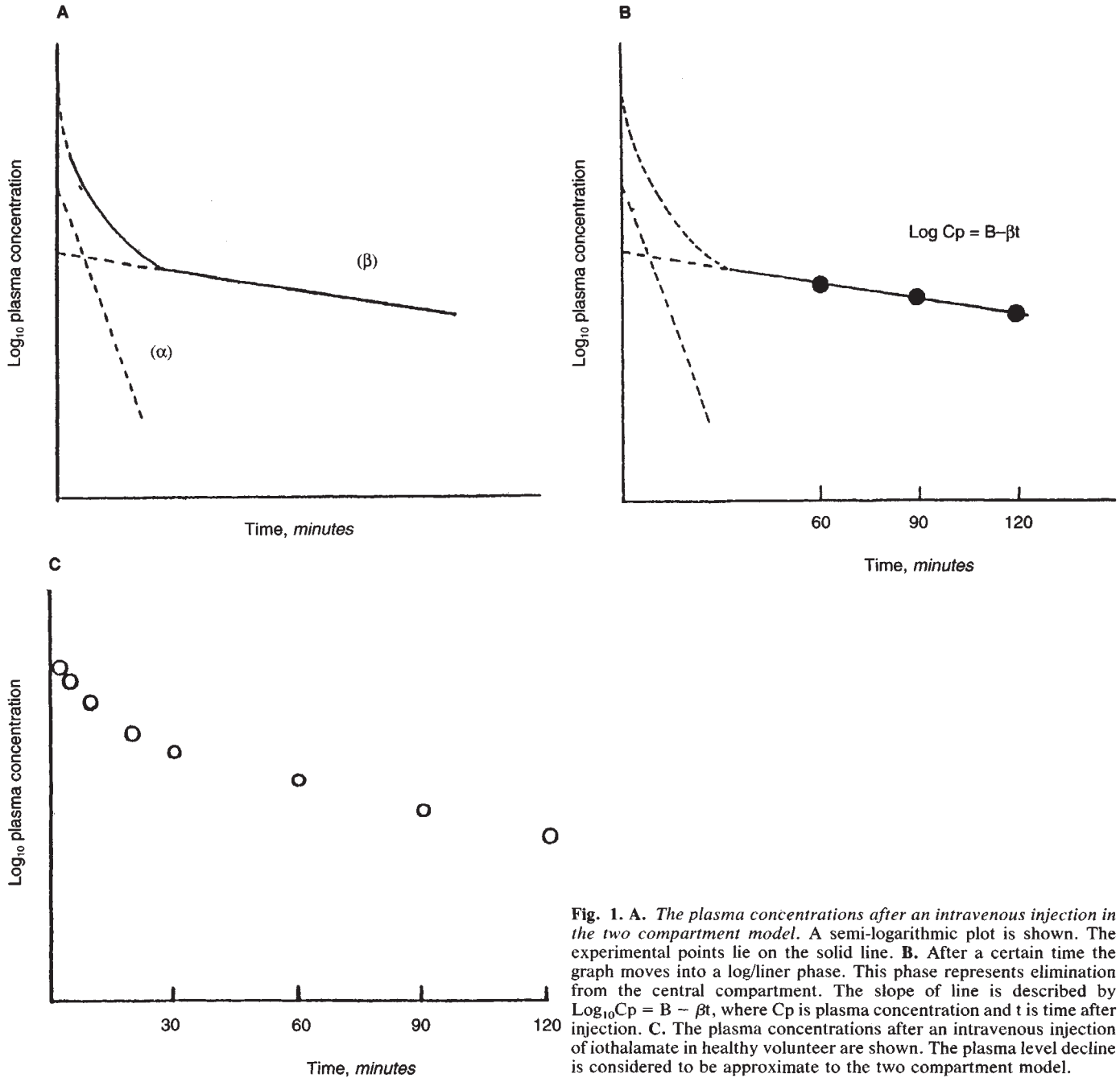

Fig. 1. A. *The plasma concentrations after an intravenous injection in the two compartment model.* A semi-logarithmic plot is shown. The experimental points lie on the solid line. **B.** After a certain time the graph moves into a log/liner phase. This phase represents elimination from the central compartment. The slope of line is described by $Log_{10}Cp = B - \beta t$, where Cp is plasma concentration and t is time after injection. **C.** The plasma concentrations after an intravenous injection of iothalamate in healthy volunteer are shown. The plasma level decline is considered to be approximate to the two compartment model.

concentrations of PAH. Therefore, in the same biological samples and the same HPLC system, one would be able to determine the clearance of iothalamate and PAH, that is, GFR and renal plasma flow, simultaneously. In our HPLC method a small sample volume with deproteinization is needed. The retention time of iothalamate was 6.0 minutes, and the coefficient of variation was 2.1%. We could rapidly and accurately determine iothalamate.

In agreement with earlier studies [1–3, 12], the renal clearance of iothalamate was correlated well with that of inulin determined simultaneously (C_{Iot}/C_{In} = 1.05 ± 0.01, r = 0.98), whereas the renal clearance of creatinine was significantly higher than that of inulin (C_{Cr}/C_{In} = 1.23 ± 0.01, r = 0.99). In the study by Odlind et al, iothalamate was not a suitable marker for glomerular filtration because of its tubular secretion [13]. It is a weak acid and may presumably be a subject for active secretion; however, from our clearance data, the gain of iothalamate clearance with a minute dose was not significant. Therefore iothalamate is considered to be an appropriate marker for glomerular filtration rate, as far as the minute dose of iothalamate is used.

We found an excellent agreement between the modified plasma clearance and the renal clearance of iothalamate (C_{Iot}-M/C_{Iot} = 0.99 ± 0.01, r = 0.98). We also found a close

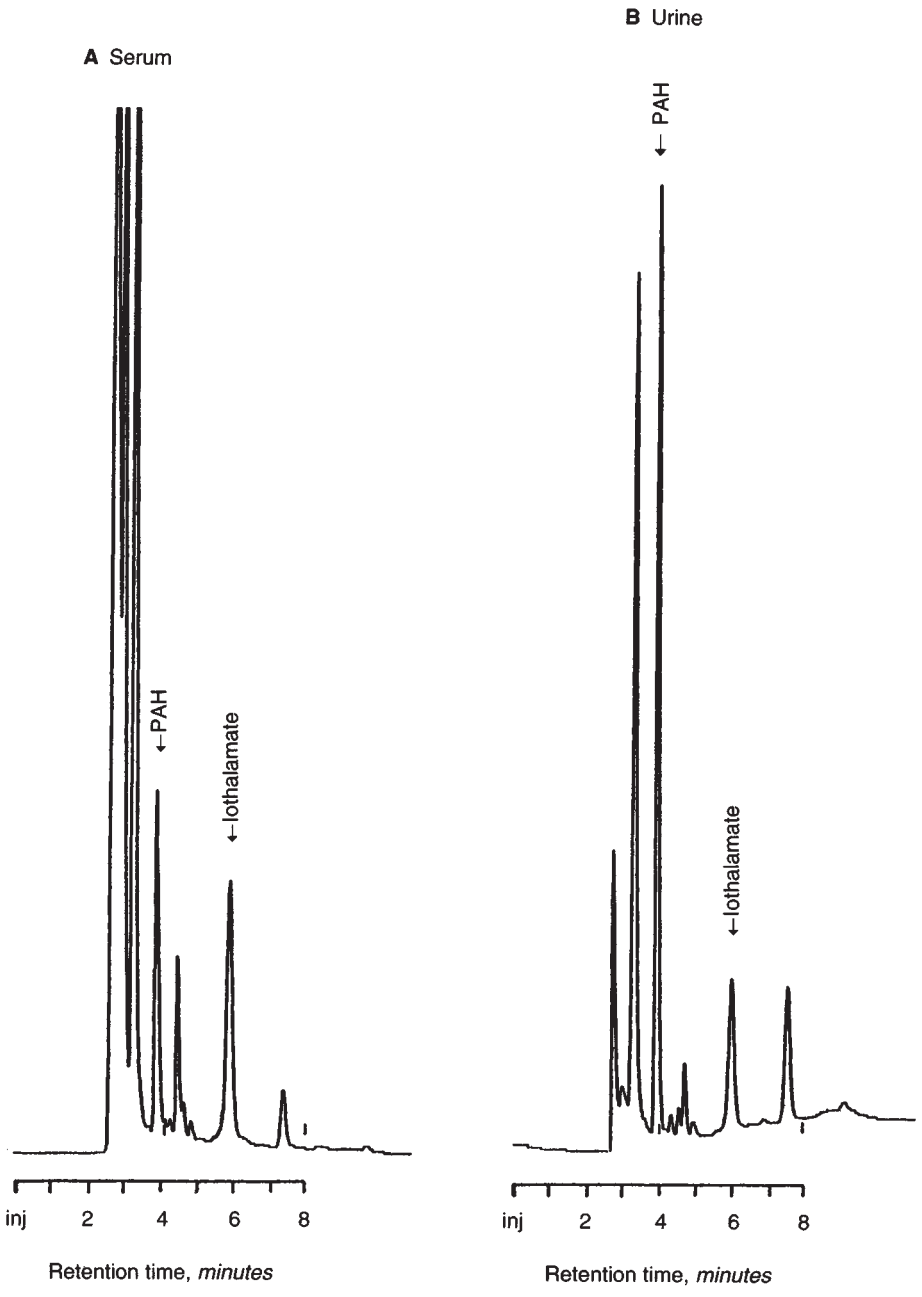

Fig. 2. *Chromatograms of (A) serum and (B) urine samples containing iothalamate (25 mg/liter and 150 mg/liter, respectively) and PAH (4 mg/dl and 80 mg/dl, respectively).*

correlation between the modified plasma clearance of iothalamate and the renal clearance of inulin (C_{Iot}-M/C_{In} = 1.05 ± 0.01, r = 0.99). The decline of the serial plasma concentrations after intravenous injection of iothalamate was considered to be in good approximation to the ideal decline in the two compartment model. We used the time at 60, 90, and 120 minutes after injection to be the sampling time of choice, because the elimination curve of iothalamate was log/linear around this time, as shown in Figure 1c.

The routine method for determination of total plasma clearance usually utilizes multiple blood samples. But the late phase of the plasma disappearance curve after single injection of iothalamate is closely related with GFR [6]. The modified plasma clearance is calculated theoretically only from three blood samples. We needed the correlation factor to determine GFR by the modified plasma clearance technique. If iothalamate should be distributed into the central compartment, in this case the plasma, and be rapidly equilibrated with the second compartment, in this case the tissue, the elimination rate of iothalamate will be determined by not only the glomerular filtration but also the ratio between the central and the second compartment. Therefore in the equilibrium phase, in this case the late phase of the plasma disappearance curve, GFR should be determined by the plasma elimination rate of iothalamate and the correlation factor, which is calculated from the intravascular space and distribution volume. This correlation factor was the same in all patients despite a wide range of renal function, but our study nevertheless showed an excellent agreement

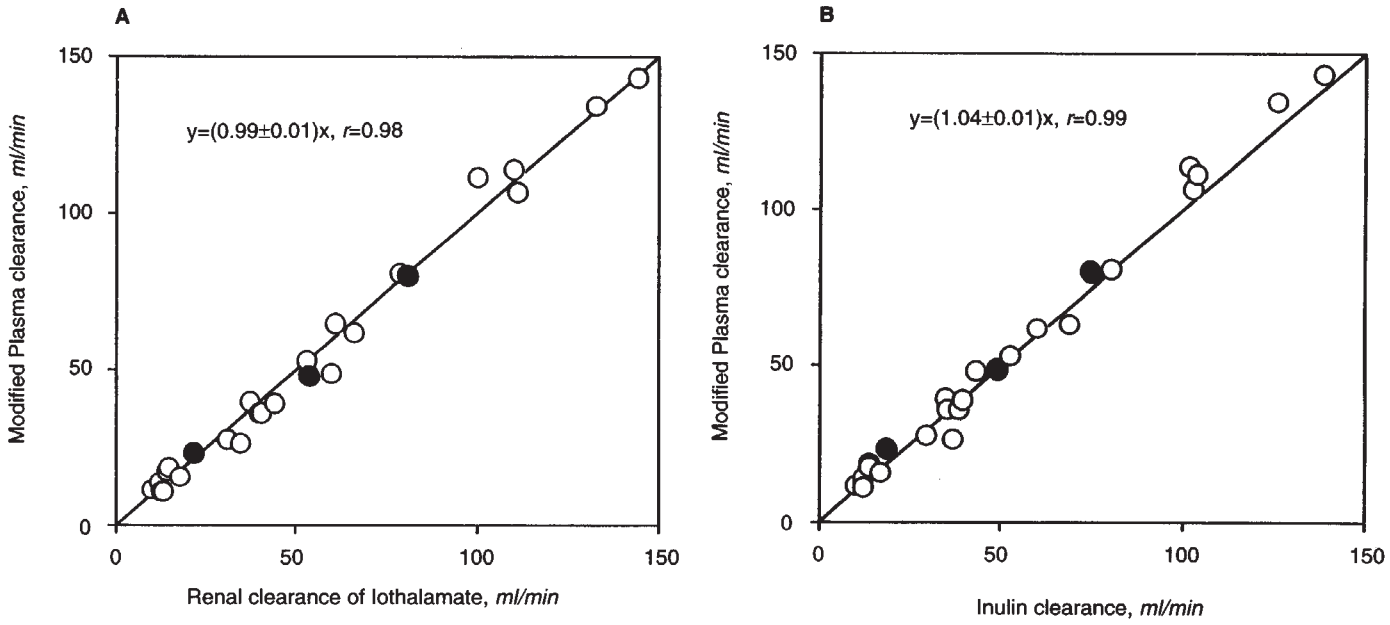

Fig. 3. *The modified plasma clearance calculated only from the late phase of the plasma decline at 60, 90, 120 minutes after an intravenous injection of iothalamate are compared with the renal clearances of iothalamate (A) and inulin (B).* The line of identity is also drawn (○: subject without edema ●: subjects with edema).

between the modified plasma clearance of iothalamate and the renal clearance of inulin irrespective of renal function. Therefore, in assessment of GFR, this correlation factor, 1.34, and a new method, the modified plasma clearance technique, would be available regardless of renal function.

Prescott, Freestone and McAuslane reported the single injection and total plasma clearance of inulin [14], but it seems to us that inulin is unsuitable for single injection technique, because of the large dose of inulin given over five minutes. In previous reports the total plasma clearance of inulin or ^{51}Cr-ethylenediaminetetraacetate exceeded the simultaneously measured renal clearance [5, 14]. As compared with the total plasma clearance, the modified plasma clearance technique does not need multiple blood sampling, for example, initial frequent sampling nor delay sampling (4 to 6 hours after injection). In addition, this technique, which does not need urine collection, will have a great advantage in patients with difficulties in urination, for example, in those with a neurogenic bladder or urinary tract obstruction.

Experimental studies utilizing the single-injection and modified plasma clearance technique will therefore have more advantage in the measurement of GFR than those utilizing the classical constant infusion technique with urine collection.

Although the correlation factor is approximately estimated from the intravascular and extravascular volume, the modified plasma clearance technique was found to be effective under different conditions, for example, edema and polycystic kidney disease. It is probably because the distribution of contrast media into the third space, such as edema and cyst, can be disregarded. The results were not different with sexes. So this technique seems to be valid without distinction of sex or disease.

In conclusion, the present study has shown excellent correlation of the modified plasma clearance of iothalamate with the renal clearance of inulin and iothalamate in patients and normal volunteers with wide range of renal function. This justifies the use of the single injection and modified plasma clearance technique in the measurement of GFR. The modified plasma clearance technique can avoid errors in urine collection and pain of multiple blood sampling. Therefore, the single injection and modified plasma clearance technique is suitable for routine measurement of GFR.

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